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The Complexity of DRw6 and DR5 Haplotypes in American Blacks Demonstrated by Serology, Cellular Typing, and Restriction Fragment Length Polymorphism Analysis

Kyung Wha Lee, Carolyn Katovich Hurley, Robert Hartzman, and Armead H. Johnson

ABSTRACT: *This study describes the diversity of DRw6 and DR5 haplotypes in the American black population using serology, cellular typing, and restriction fragment length polymorphism (RFLP) analysis. DRw6 (DRw13 and DRw14) and DR5 (DRw11 and DRw12) haplotypes are observed at a high frequency in this population (DRw6: 32%, DR5: 30%). Many of these haplotypes express undefined HLA-D specificities and unusual DQ and DRw52 associations which previously have not been well characterized or reported (e.g., DRw13, DQw5, DRw52c, D-; DRw13, DQw2, DRw52a, D-; DRw11, DQw5, DRw52c, D-). Serologic analysis of class II alleles in American blacks suggests the presence of DRw13, DRw11 and DQw6 allelic variants and demonstrates the difficulty in defining DRw6 and DR5 in this population. The class II genes from four American black families expressing many of the novel DRw13, DRw14, DRw11, and DRw12 haplotypes defined by serology and mixed leukocyte culture were further characterized by RFLP analysis. The data presented here along with other published data identify at least eight DRw13 haplotypes (DRw13A–DRw13H) in the human population. Five of these haplotypes exhibit an undefined HLA-D specificity. Three DRw14 haplotypes (DRw14A–DRw14C) and eight DR5 haplotypes (DRw11A–DRw11E and DRw12A–DRw12C) were also identified. The novel DRw6 and DR5 haplotypes observed in American blacks may arise from differences in DRB1, DQA1, and DQB1 genes as well as from differences in the combinations of alleles of these genes encoded by a haplotype. The serologic and RFLP analyses suggest that some DRw13 and DRw11 haplotypes represent transitional steps between DRw13 and DRw11 in the evolutionary pathway which generated the DRw52 family.*

ABBREVIATIONS

B-LCL	B-lymphoblastoid cell line	IHWS	International Histocompatibility Workshop
FCS	fetal calf serum		
HTC	homozygous typing cell	RFLP	restriction fragment length polymorphism

INTRODUCTION

The class II region of the human major histocompatibility complex encodes highly polymorphic heterodimeric (α and β) cell surface glycoproteins (DR, DQ, and

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DP), which function in the induction of the normal immune response [1] and are responsible in part for allograft rejection [2] and autoimmune disease susceptibility [3]. The polymorphic residues of the class II polypeptides are localized to the amino terminal domains and are clustered into variable regions. In the predicted three-dimensional structure of the class II molecule, the majority of the polymorphic sites reside in the antigen-binding T-cell receptor recognition site suggesting the importance of these residues in controlling antigen recognition and allograft rejection [4].

HLA-D regions that encode molecules carrying DRw52 serologic determinants share general structural features suggesting a common origin. The DR subregion of most of these haplotypes encodes one nonpolymorphic α gene (DRA) and three DRβ genes [5]. One highly polymorphic β gene, DRB1, controls the expression of molecules exhibiting DR3, 5, w6, and w8 serologic specificities [6]. A second, less polymorphic β gene, DRB3, controls the expression of DRw52a, 52b, and 52c molecules [7]. The third is a pseudogene, DRB2. The DQ subregion encodes one set of expressed α and β genes (DQA1 and DQB1) controlling the expression of molecules with DQw1–w4 serologic specificities as well as nonexpressed genes (DQA2 and DQB2) [6]. The DRB1, DRB3 and DQ alleles are often nonrandomly associated with each other forming haplotypes, a phenomenon called linkage disequilibrium. For example, in northern European populations, DRw6 alleles tend to be inherited with DQw1 and DR5 alleles tend to be inherited with DQw3. DRw6 and DR5 alleles are always associated with a DRw52 allele.

The polymorphism of the class II molecules was originally defined using serology and mixed leukocyte culture. Historically, haplotypes expressing DRw6 and DR5 alleles have been difficult to define with serologic reagents [8–11]. For example, DRw6 alleles were frequently defined by their association with DRw52 and DQw1 and by their patterns of reactivity with multispecific alloantisera. During the Ninth and Tenth International Histocompatibility Workshops (IHWS), DRw6 and DR5 were redefined and subdivided in terms of serologic (DR and DQ) and cellular (HLA-D) specificities. At least four well-defined haplotypes were defined for DRw6: DRw13, DQw6, Dw18; DRw13, DQw6, Dw19; DRw14, DQw5, Dw9; and DRw14, DQw7, Dw16 [8,9]. (DQw5 and DQw6 are subdivisions of DQw1; DQw7 is a subdivision of DQw3.) At least two well-defined haplotypes were defined for DR5: DRw11, DQw7, Dw5 and DRw12, DQw7, DwDB6 [10,11]. Nevertheless, the distinctions among DRw13, DRw11, and DRw12 have remained unclear when cells express either variant DR alleles or the more uncommon DR/DQ allele associations (e.g., DRw13, DQw7; DRw11, DQw5; DRw12, DQw5).

The difficulty in defining DRw6 and DR5 is particularly evident when non-Caucasian populations have been studied. This problem is acute in the American black population where DRw6 and DR5 are observed at a high frequency. The problem is compounded by this high frequency since many individuals express two DRw6 and/or DR5 haplotypes. Many of the DRw6- and DR5-related haplotypes in blacks have not been previously described and express undefined HLA-D specificities and unusual DR/DRw52/DQ associations. Alloantisera were used to differentiate DRw13, DRw11 and DQw6 variants and to demonstrate the complexity of the serologic typing of these HLA alleles. To analyze these DRw6 and DR5 haplotypes further at the genomic level, four American black families expressing six DRw6 haplotypes and five DR5 haplotypes were selected for restriction fragment length polymorphism (RFLP) analysis. Using the Southern hybridization technique with locus-specific probes, DNA restriction fragments from these individuals were compared to DRw6 and DR5 homozygous typing

cells (HTCs) defining the more common DRw6 and DR5 haplotypes found in northern European populations. The serologic, cellular, and RFLP data have identified at least 11 DRw6 haplotypes (DRw13A–DRw13H and DRw14A–DRw14C) and eight DR5 haplotypes (DRw11A–DRw11E and DRw12A–DRw12C) in the human population. As compared to previously defined DRw6 and DR5 haplotypes, the novel DRw6 and DR5 haplotypes observed in American blacks may arise from differences in DRB1, DQA1, and DQB1 genes as well as from differences in the combinations of alleles of these genes encoded by a haplotype. The evolutionary relationship between DRw13 and DRw11 is clearly evident in this analysis.

MATERIALS AND METHODS

HLA typing. Lymphoctyes were separated from peripheral whole blood on a Ficoll-Hypaque gradient. Lymphocytes were typed for HLA-A, -B, -C, -DR, and -DQ using the 10th IHWS alloantisera set as well as a set of alloantisera selected from our own collection and from colleagues. HLA-A, B, C specificities were determined as described by Sullivan and Amos [12]. For the determination of DR and DQ specificities, immunoglobulin-positive cells were selected positively by using an affinity purified goat anti-human F(ab)$_2$ monolayer after carbonyl iron treatment to remove macrophages. Immunoglobulin-positive B cells were eluted and the DR and DQ specificities analyzed by using a microcytotoxicity assay [13].

HLA-D region antigens were defined in primary mixed lymphocyte cultures utilizing HTCs analyzed in IHWS and local typing cells [14]. Fifty thousand responder cells were combined with 5×10^4 gamma-irradiated stimulator cells in triplicate cultures in 96-well U-bottom plates. After 4 days, each culture was pulsed overnight with 1 μCi (5 Ci/mmol) of [^{3}H]thymidine and harvested onto glass fiber filters [15]. Radiolabel incorporation was monitored by liquid scintillation counting. Data were statistically analyzed using the 75th percentile double normalized value according to Ryder et al. [16].

Lysostripping. The lysostripping technique was employed to determine which class II molecule reacted with a given alloantiserum. Purified B lymphocytes or B-lymphoblastoid cell lines (B-LCLs) were reacted with a monoclonal antibody specific for either DR (L203), DQ (33.1), DP (B7/21) [17], or β-2 microglobulin (MB40.5) (Atlantic Antibodies, Scarborough, ME) for 30 min at 24°C to remove (i.e., lysostrip) the given molecular subset of molecules from the cell surface, temporarily rendering the cell unsusceptible to lysis by a second antibody directed against that same molecular subset. The cells were washed twice in RPMI 1640 plus 10% fetal calf serum (FCS) and the cell count was adjusted to 2×10^6/ml. The cells were immediately tested by a standard microcytotoxicity assay using dilutions of the test antiserum as well as using dilutions of alloantisera known to react with DR, DQ, or DP as positive controls for the lysostripping procedure.

DRw52 subtyping. DRw52 subtypes were determined by a combination of RFLP analysis and T-cell clone typing. Haplotypes expressing DRw52b carry a 12-kb TaqI/DRB3 DNA restriction fragment while haplotypes expressing DRw52a or DRw52c carry a 9.6-kb TaqI/DRB3 fragment [18]. T-cell clones specific for Dw25 (DRw52b) and Dw26 (DRw52c), generated locally, were characterized against the 10th IHWS reference panel of B-LCLs. DRw52a was assigned by the presence of a 9.6-kb TaqI/DRB3 fragment and/or the lack of stimulation of either Dw25- or Dw26-specific T-cell clones. Methods for generating T-cell clones have been previously described [19].

TABLE 1 American black families used in the RFLP analysis

Family 004	2015	a	A2	C-	B45	(w6)	D-	DRw11	DRw52c	DQw5	DPw2
		b	Aw33	Cw3	B27	(w4)	D-	DRw11	DRw52b	DQw7	DPw3
	1183	c	A30	Cw8	B7	(w6)	NT	DRw12	DRw52a	DQw5	DPw1
		d	A32	Cw8	Bw64	(w6)	D-	DRw13	DRw52a	DQw7	DPw1
	1179	a/b	A2	C-	B45	(w6)	D-	DRw11	DRw52b	DQw7	DPw3
		c	A30	Cw8	B7	(w6)	NT	DRw12	DRw52a	DQw5	DPw1
	1180	a	A2	C-	B45	(w6)	D-	DRw11	DRw52c	DQw5	DPw2
		c	A30	C-	B7	(w6)	NT	DRw12	DRw52a	DQw5	DPw1
	1181	b	Aw33	Cw3	B27	(w4)	D-	DRw11	DRw52b	DQw7	DPw3
		d	A32	Cw8	Bw64	(w6)	D-	DRw13	DRw52a	DQw7	DPw1
Family 011	2011	a	A11	Cw7	B18	(w6)	Dw18	DRw13.1	DRw52a	DQw6	DPw4
		b	A2	Cw4	B35	(w6)	Dw19	DRw13.2	DRw52c	DQw6	DP-
	2009	a	A11	Cw7	B18	(w6)	Dw18	DRw13.1	DRw52a	DQw6	DPw4
		c	Aw68	Cw6	Bw58	(w4)	D-	DRw11	DRw52b	DQ-	DP-
	2013	b	A2	Cw4	B35	(w6)	Dw19	DRw13.2	DRw52c	DQw6	DP-
		d	A23	Cw4	Bw53	(w4)	D-	DRw14	DRw52a	DQw5	DP-
	2012	a	A11	Cw7	B18	(w6)	Dw18	DRw13.1	DRw52a	DQw6	DPw4
		d	A23	Cw4	Bw53	(w4)	D-	DRw14	DRw52a	DQw5	DP-
Family 014	2708	c	Aw68	Cw6	Bw57	(w4)	D-	DRw13	DRw52a	DQw2	DPw1
		d	Aw68	Cw6	Bw58	(w4)	NT	DRw12	DRw52b	DQw5	DP-
	1066	a	A2	C-	B45	(w6)	D-	DRw8		DQw7	DPw1
		d	Aw68	Cw6	Bw58	(w4)	NT	DRw12	DRw52b	DQw5	DP-
	2710	b	Aw36	Cw7	Bw58	(w4)	D-	DRw18	DRw52a	DQw4	NT
		d	Aw68	Cw6	Bw58	(w4)	NT	DRw12	DRw52b	DQw5	NT
Family 021	1129	c	A11	Cw3	Bw55	(w6)	Dw1	DR1		DQw5	DPw4
		d	Aw34	Cw6	Bw58	(w4)	D-	DRw11	DRw52b	DQw7	DP-
	1124	b	Aw68	Cw8	Bw65	(w6)	D-	DRw13	DRw52b	DQw7	DP-
		c	A11	Cw3	Bw55	(w6)	Dw1	DR1		DQw5	DPw4
	1127	a	A30	Cw4	Bw53	(w4)	D-	DRw8		DQw7	DPw1
		c	A11	Cw3	Bw55	(w6)	Dw1	DR1		DQw5	DPw4
	1125	b	Aw68	Cw8	Bw65	(w6)	D-	DRw13	DRw52b	DQw7	DP-
		d	Aw34	Cw6	Bw58	(w4)	D-	DRw11	DRw52b	DQw7	DP-
	2717	a	A30	Cw4	Bw53	(w4)	D-	DRw8		DQw7	DP-
		c	A11	Cw3	Bw55	(w6)	Dw1	DR1		DQw5	DPw4

Only the family members used in the RFLP study are listed. Since cells expressing Dw18 and Dw19 are known to express different DRB1 alleles, these DR alleles have been designated DRw13.1 and DRw13.2 [20,21]. The haplotypes of the family members used in the serology study described in Tables 3 and 4 and not listed here are: family 004: 1195 (a/c), 1193 (b/a/d), 1198 (b/c); family 014: 2704 (a/c); family 021: 1126 (b/c).

A specificity undefined by our reagents is indicated as -. NT, not tested.

B-LCLs used in RFLP analysis. B-LCLs (Table 1) were established by transforming peripheral blood lymphocytes with Epstein-Barr virus [22]. Purified B lymphocytes enriched by using magnetic beads coated with monoclonal antibodies to remove T lymphocytes [CD7 (T3-3A1)] [23] and monocytes [CD11 (OKM1)] [24] were incubated with Epstein-Barr virus for 2 hr at 37°C and plated in 24-well plates (0.25×10^6 cell/well) in RPMI 1640 supplemented with 15% FCS, 15

mM HEPES, and 50 μg/ml gentamycin. After transformation, cell concentrations were maintained at 3 to 9 × 10^5 cells/ml. Transformed lines were HLA typed after transformation to confirm their identity. HLA-D region HTCs used in the study were: APD (DRw13, DRw52b, DQw6, Dw18), HHK (DRw13, DRw52a, DQw6, Dw18), ARENT (DRw13, DRw52a, DQw6, Dw18), SLE-005 (DRw13, DRw52c, DQw6, Dw19), EK/OH (DRw14, DRw52b, DQw5, Dw9), AMALA (DRw14, DRw52a, DQw7, Dw16), IDF (DRw11, DRw52b, DQw7, Dw5), and BM16 (DRw12, DRw52b, DQw7, DwDB6). These B-LCLs were obtained from the NIGMS Human Genetic Mutant Cell Repository, Camden, NJ, and the 10th IHWS panel.

Southern hybridization analysis. DNA (10 μg), prepared as previously described [25], was digested with restriction endonucleases (TaqI, PvuII, BamHI, BglII, EcoRI, HindIII, and PstI) in the appropriate buffers as directed by the manufacturer (New England BioLabs, Beverly, MA). Restriction endonuclease-digested DNA was electrophoresed in 0.8% agarose gels in Tris-acetate-EDTA buffer and was transferred to Genetran paper (Plasco, Woburn, MA) [26]. The filters were hybridized with 10^7 cpm radiolabeled heat denatured probe as previously described [25] and specific DNA fragments were detected by autoradiography. Densitometer scanning was used to estimate the intensity of hybridization of the fragments. Families were used to allow the assignment of DNA fragments to haplotypes based on segregation analysis. DRw6 and DR5 HTCs were included for comparison.

cDNA probes encoding human DRβ, DRα, DQβ, and DQα chains have been described [25,27]. The DRβ short (DRβ 3′) probe containing only the 3′ untranslated region was obtained from the full-length clone by digestion with HindIII and EcoRI. Although this 3′ probe used in the DRβ RFLP study hybridizes to only the 3′ untranslated region of the three DRβ genes and not to the coding regions, polymorphic fragments can be identified which correlate with serologic polymorphism [28,29]. In addition, using the 3′ probe, it was possible to estimate the number of DRβ loci in each haplotype and to simplify the RFLP patterns in the individuals studied. The DQα and DQβ probes detect all of the DQ genes [25]. DNA probes were labeled with α [^{32}P]-deoxycytidine triphosphate (3000 Ci/mmol; NEN, Boston, MA) using random hexamer priming [30].

RESULTS

DRw6 and DR5 haplotypes are found at a high frequency in the American black population. A total of 204 American black individuals from the Washington DC area, including 105 unrelated individuals and 17 families, have been characterized for HLA-A, -B, -C, -D, -DR, and -DQ. Thirty-two percent of unrelated individuals express DRw6 (26% express DRw13 and 6% express DRw14) and 30% of unrelated individuals express DR5 (25% express DRw11 and 5% express DRw12). Fifty-nine percent of the DRw6-unrelated individuals and 82% of the DR5-unrelated individuals do not express an HLA-D specificity defined by our reagents (i.e., not Dw18, Dw19, Dw9, Dw16, or Dw5). In comparison, only 8% of the DRw6-positive Caucasoids and 18% of DR5-positive Caucasoids on our local cell panel express an undefined D specificity.

Some DRw13.D- haplotypes cannot be distinguished from DRw11 haplotypes when DRB1 DNA restriction fragments are analyzed. Based on the population analysis, four American black families (Table 1) expressing six DRw6 haplotypes (five DRw13 and one DRw14) and five DR5 haplotypes (three DRw11 and two

TABLE 2 Summary of the RFLP analysis of DRw6/5-related haplotypes in American black families compared to related HTCs

DRw	13							14			11				12		
Dw	18		19	-	-	-	-	9	16	-	5	-	-	-	086	NT	NT
DQw	6		6	5	2	7	7	5	7	5	7	5	-	7	7	5	5
DRw52	b	a	c	c	a	a	b	b	a	a	b	c	b	b	b	a	b
HTC	APO	HHK	SLE					EK/OK	AAALA		IDF				BM16		
RFLP Family # & Cell / Probe / Enzyme		011a 2012	(0110) 2013	1083	014c 2708	004d 1181	021b 1125			011d 2013		004a 1180	011c 2009	004b 021d 1181		004c 1180	014c 1066
OR beta TaqI	6.8 12	6.8 9.6	6.8 9.6	6.8 9.6	5.9 9.6	5.9 9.6	5.9 12	6.8 12	6.8 9.6	6.8 9.6	5.9 12	5.9 9.6	5.9 12	5.9 12	4.1 12	4.1 9.6	4.1 12
OR alpha Bgl II	4.5	4.2	(4.5) 4.2	4.5	4.2	4.2	4.5	4.5	4.2	4.2	4.5	4.5	4.5	4.5	4.5	4.2	4.5
DQ beta BamHI	3.2 7.5	3.2 7.5	7.5	NT	4.5	4.1	4.1	3.4	4.1	3.4	4.1	3.4	4.5	4.1	4.1	3.4	3.4
DQ beta TaqI	3.0 2.7	3.0 2.7	5.7	NT	7.2	-	-	5.7	-	5.7	-	5.7	3.9	-	-	5.7	5.7
DQ alpha EcoRI	19	19	19	NT	10 7.8	15	NT	NT	NT	19	15	19	7.8	15	NT	19	19
DQ alpha HindIII	8.0	8.0	8.0	NT	4.8 4.2	6.0	NT	NT	NT	8.0	6.0	8.0	4.8	6.0	NT	8.0	8.0
DQ alpha PstI	15	15	15	NT	4.2 2.8 2.3	4.2	NT	NT	NT	15	4.2	15	2.8 2.3	4.2	NT	15	15
DQ alpha TaqI	6.5 2.0	6.5 2.0	5.9 2.0	NT	5.1 1.8	4.3 2.0	4.3 1.8	3.4 2.4 2.0	4.3 1.8	3.4 2.4 2.0	4.3 1.8	5.9 2.0	5.1 1.8	4.3 1.8	4.3 1.8	3.4 2.4 1.8	3.4 2.4 1.8
Class II Haplotype	A	B	C	D	E	F	G	A	B	C	A	B	C	D	A	B	C

Sizes of polymorphic DNA restriction fragments associated with the haplotypes are listed. Family number, haplotype, and a representative cell are indicated for each column (e.g., 011a = "a" haplotype of family 011 expressed by cell 2012). Haplotypes are defined as DRw13A-DRw13G, DRw14A-DRw14C, DRw11A-DRw11D, and DRw12A-DRw12C based on serology, HTC typing, and RFLP analysis. Serologically defined haplotypes DRw13H and DRw11E are not listed in this table since sufficient family members were not available for RFLP analysis. HTC ARENT gives the same restriction fragment pattern as HTC HHK with the exception of the DQA2 gene. The DRA restriction fragment from SLE differs from that found in family 011 [indicated by () in this table]. A TaqI/DQβ fragment, observed at 4.7 kb, is shared by all cells. Based on intensity, DQw7-positive cells appear to carry a second fragment also migrating at 4.7 kb (indicated by *).

NT, not tested.

DRw12) were chosen for RFLP study. Southern blot hybridization was used to identify polymorphic DNA restriction fragments associated with several unusual DRw6 and DR5 haplotypes found in the families, to measure the allelic polymorphism within the DRw6 and DR5 haplotypes and to analyze evolutionary relatedness among class II alleles. The RFLP data will be discussed in terms of haplotypes (e.g., DRw13A, DRw11B) as defined by serology and cellular and RFLP analysis (Table 2).

In the comparison of DRβ gene fragments among families and HTCs, at least two different DRw13-associated DRB1 gene fragments were detected (Fig. 1, Table 2). The first fragment, a 5.9-kb fragment, is associated with the novel DRw13 haplotypes found in American blacks (haplotypes DRw13E, DRw13F and DRw13G) and with the DRw11 haplotypes. (This fragment is also exhibited by cells expressing DRw18 [27].) The second fragment, a 6.8-kb fragment found in all of the DRw13 HTCs (haplotypes DRw13A, DRw13B, and DRw13C) and DRw14 HTCs (haplotypes DRw14A and DRw14B), is associated with DRw13B, DRw13C, DRw13D, and DRw14C haplotypes expressed by the families. (DRw13A is not expressed by the families under study.)

All of the DRw11 and DRw12 haplotypes from the American black families carry the same sized DRB1 fragments (5.9 kb for DRw11 and 4.1 kb for DRw12)

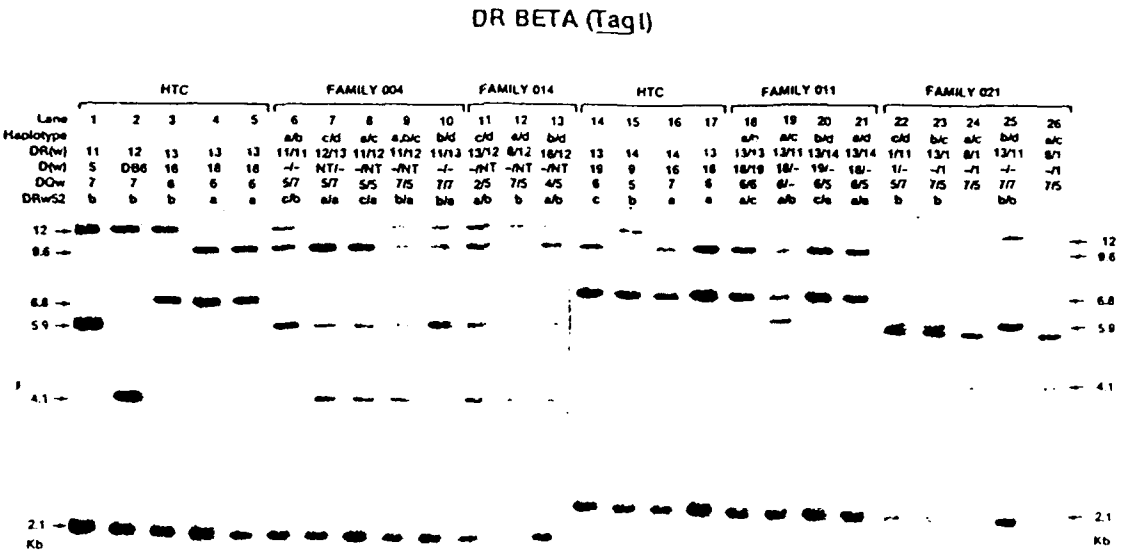

FIGURE 1 Southern hybridization analysis of DRw6/5-related haplotypes in several American black families. Genomic DNAs were digested with TaqI and probed with a DRβ 3′ probe. HTCs: lane 1 = IDF (DRw11,Dw5), lane 2 = BM16 (DRw12, DwDB6), lane 3 = APD (DRw13, Dw18), lane 4 = HHK (DRw13, Dw18), lane 5 = ARENT (DRw13, Dw18), lane 14 = SLE-005 (DRw13, Dw19), lane 15 = EK/OH (DRw14, Dw9), lane 16 = AMALA (DRw14, Dw16), lane 17 = ARENT (DRw13, Dw18). Family 004: lane 6 = 2015, lane 7 = 1183, lane 8 = 1180, lane 9 = 1179, lane 10 = 1181. Family 014: lane 11 = 2708, lane 12 = 1066, lane 13 = 2710. Family 011: lane 18 = 2011, lane 19 = 2009, lane 20 = 2013, lane 21 = 2012. Family 021: lane 22 = 1129, lane 23 = 1124, lane 24 = 1127, lane 25 = 1125, lane 26 = 2717. Families were used to allow the assignment of DNA fragments to haplotypes based on segregation analysis. For example, in family 004 (lanes 6 through 10), the father (2015, a/b) (lane 6) carries a DRw52b(DRB3)-associated 12-kb fragment [18] not observed in the mother (1183, c/d) (lane 7). This fragment is observed in siblings expressing the b haplotype and not in a sibling expressing the a haplotype (lane 8), thus assigning this fragment to the b haplotype. The remaining haplotypes in family 004 encode DRw52a (c,d haplotypes) and DRw52c (a haplotype) alleles, as defined by T-cell clone typing, associated with a 9.6-kb fragment. The dosage of the DRw52a/c alleles is reflected in the intensity of the DRw52a/c-associated 9.6-kb band. The summation of the intensity of the 12- and 9.6-kb fragments from the father is similar to the mother and sibling 1180 carrying two doses of the 9.6-kb fragment. In a similar manner, the DRB1 fragments at 4.1 and 5.9 kb have been assigned to the c (4.1-kb) and a/b/d (5.9-kb) haplotypes. The shared 2.1-kb fragment carried by all HTCs and individuals expressing DRw52 haplotypes is likely the DRB2 pseudogene.

as the reference HTCs. Although several additional restriction enzymes (EcoRI, HindIII, PstI, and BamHI) were used in a pilot study using family 004 which expresses DRw13, DRw12 and two DRw11 haplotypes, none of the enzymes revealed the polymorphism present within the DRw6 and DR5 haplotypes as detected with TaqI (data not shown).

DRw13 and DRw11 variants are observed using a series of alloantisera. The serologic reaction patterns for DRw13, DRw11, and DRw12 for the four black families studied by RFLP analysis, as well as unrelated black individuals, are defined in Tables 3 and 4. Cells are grouped based on their HLA-D type, DR and DQ serologic patterns, and their family association. The serology was used to identify variant alleles and to demonstrate the difficulty in defining DRw6 and DR5 alleles in the American black population.

DRw13 is clearly defined by IHWS sera 1133, 1126, 1124 (Table 3) except

TABLE 3 Serologic reaction patterns for DRw13, DRw12, DQw1, DQw5, and DQw6 alloantisera for family members and representative unrelated American blacks.

CELLS							ANTISERA						
							DRw11	DRw12	DRw13	DQw1	DQw6	DQw5	
GROUPS	FAMILY	ID	HLA TYPE Dw	DRw	DRw	DQ	* * C G G H H S 1 1 C D A E 1 1 4 D C W L 1 1 3 U 0 1 B 3 4 7 C L L Y	* * 9 9 9 0 9 5 9 0	* * * 1 1 1 1 1 1 3 2 2 3 6 4	* * 1 1 1 1 5 6 3 2	I A 7 6 P A	L O B E 1 E 1 2 L B 1 1 Y C	
		44	18,2	13.1,15	52a	w6	1 1 4 2 1 1 1	1 1	6 6 6	6 6	8	1 1 1	
1	011	2012	18,-	13.1,14	52a,52a	w6,w5	1 1 1 1 1 1 1	1 1	8 6 6	6 6	8	4 6 6	
	011	2009	18,-	13.1,11	52a,52b	w6,-	8 8 8 8 8 8 8	1 1	8 6 6	6 6	8	1 1 6	
2	011	2013	19,-	13.2,14	52c,52a	w6,w5	1 1 1 1 1 1 1	1 1	8 6 4	6 6	2	8 6 6	
		265	19,2	13.2,15	52c	w6,w6	1 1 2 1 1 1 1	1 1	8 6 4	6 8	1	1 1 1	
3	•	1233	-,-	13,1	52c	w6,w5	1 1 4 1 1 1 1	1 1	8 6 6	8 8	8	8 8 8	
		1235	-,-	13,7	52c,53	w5,w2	1 1 2 2 4 1 2	1 1	8 8 8	6 8	1	6 4 6	
4		1242	-,NT	13,12	52c	w5	1 1 1 2 1 1 1	6 6	6 4 6	6 6	1	0 4 6	
	•	1083	-,-	13,9	52c,53	w5,w2	1 1 2 1 1 2 1	2 2	8 8 6	6 8	1	8 8 8	
		1560	-,-	13,17	52c,52b	w5,w2	1 1 1 1 1 1 1	1 1	8 8 6	6 6	1	0 6 6	
	021	1126	-,1	13,1	52b	w7,w5	1 1 2 1 1 1 1	1 1	8 8 8	8 6	1	6 6 8	
5	021	1125	-,-	13,11	52b,52b	w7,w7	8 8 8 8 8 4 2	1 1	8 8 8	1 1	1	1 1 1	
	021	1129	-,1	11,1	52b	w7,w5	8 8 8 8 8 2 1	1 1	8 8 8	8 8	1	6 6 8	
	014	2710	NT,-	12,18	52b,52a	w5,w4	1 1 1 1 1 1 1	8 8	1 1 4	8 6	1	6 6 6	
	014	1066	NT,-	12,8	52b	w5,w7	1 1 1 1 1 1 1	8 8	4 4 4	8 6	1	8 6 6	
	014	2708	-,NT	13,12	52a,52b	w2,w5	1 1 8 8 6 1 2	8 8	8 6 8	6 8	1	8 8 8	
	014	2704	-,-	13,8	52a	w2,w7	1 1 8 8 1 1 1	1 1	6 2 6	1 1	1	1 1 1	
6	004	1183	-,NT	13,12	52a,52a	w7,w5	1 1 8 8 4 1 1	8 8	8 8 8	0 8	1	8 8 8	
	004	1193	-,-	13,11	52a,52c	w7,w5	8 8 8 8 8 8 8	1 1	8 8 8	8 8	1	6 4 8	
	004	1198	-,NT	11,12	52b,52a	w7,w5	8 8 8 8 8 8 8	8 8	8 8 8	8 8	1	8 8 8	
	004	1181	-,-	13,11	52a,52b'	w7,w7	8 8 8 8 8 8 8	1 2	8 8 8	1 1	1	1 1 1	
	004	1195	-,NT	11,12	52c,52a	w5,w5	8 8 8 8 8 8 8	8 8	1 1 6	8 8	1	6 6 8	

HLA specificities are listed in the order of haplotype association for family members (see Table 1) and other genotyped (°) individuals. Additional family members are included to allow identification of the serologic pattern by segregation analysis.

- indicates an HLA specificity undefined by our reagents. NT, not tested. *, 10th IHWS alloantisera. The numbers in the body of the table indicate the strength of the cytotoxic reaction (8 = 85%–100% cytotoxicity; 6 = 51%–84%; 4 = 21%–50%; 2 = 11%–20%; 1 = 0%–10%; 0 = not tested).

when the sera are tested with cells which express the DRw11v specificity as described below. A variant DRw13 allele may be expressed by cells in group 6 (Table 3). In addition to reactivity with DRw13 alloantisera, these cells react strongly with two DRw11 alloantisera (CC437 and DUC) and weakly with one DRw11 alloantisera (GHDCOL), suggesting that these cells may express a DRw13 variant (DRw13v1) which shares serologic determinants with DRw11. The subdivision of DRw13 by serology parallels the two DRw13 alleles defined by RFLP analysis of DRB1 fragments, with the exception of the DRw13G haplotype.

DRw11 can be clearly defined using alloantisera 1113, 1114, CC437, DUC, and GHDCOL (Table 4). Two alloantisera, GHAWIL and SELBY, reproducibly do not react with some DRw11-positive cells discriminating group 1 (negative with the alloantisera) from group 2 (positive with the alloantisera). Since DRw11-positive cells in group 2 are associated with DQw5 or DQw7, this reactivity pattern is most likely derived from detection of the DRw11 molecule and suggests the presence of a DRw11 variant (DRw11v) in group 1. This variant DRw11

TABLE 4 Serologic reaction patterns for family members and representative unrelated American blacks who illustrate the two DRw11 subtypes detected by serology.

			CELLS				ANTISERA	
							DRw11	DRw13
GROUPS	FAMILY	ID	HLA TYPE Dw	DRw	DRw	DQ	* * C G G H H A > E D A E 1 1 C D A E 1 1 4 D C W L 1 1 3 U 0 1 B 3 4 7 C L L Y	* * * 1 1 1 1 1 1 3 2 2 3 6 4
	021	1125	-,-	11,13	52b,52b	w7,w7	8 8 8 8 8 4 2	8 8 8
1	021	1129	-,1	11,1	52b	w7,w5	8 8 8 8 8 2 1	8 8 8
		1555	-,-	11,8	52b	w7,w6	8 8 8 8 8 1 1	8 8 8
	•	1563	-,-	11,18	52b,52a	w7,w4	8 8 8 8 8 1 1	8 8 6
		1553	5,-	11	52b	w7,w6	8 8 8 8 8 8 8	6 2 1
2		1147	NT	11,7	52b,53	w7,w8	8 8 8 8 8 8 8	1 1 1
	004	1195	-,NT	11,12	52c,52a	w5,w5	8 8 8 8 8 8 8	1 1 6

See Table 3 for explanation of terminology

pattern was confirmed using an additional 10 unrelated DRw11 black individuals (data not shown). HLA-DRw11v-positive cells (Table 4, group 1) also exhibit positive reactivity with the DRw13 alloantisera. This cross-reactivity suggests that the DRw11v carries DRw13 epitopes.

Cell 1198 (DRw11,w12) (Table 3) does not fit into any one of the two DRw11 serologic patterns being detected with both the DRw11v (GHAWIL, SELBY) and the DRw13 alloantisera and, thus, represents a third serologic pattern. Two alternative explanations may explain this reactivity: (1) cross-reactivity of technique-dependent alloantisera with a cell heterozygous for two serologic specificities similar to DRw13 (DRw11,w12) or (2) a third DRw11 serologic pattern defining another DRw11 variant. Since all of the haplotypes in this family are DR5 or DRw6, segregation analysis is uninformative. Indication that the first explanation may be true is the weak reaction observed with cell 1125 (DRw11v,w13) with the DRw11v alloantisera and cell 1195 (DRw11,w12) with DRw13-specific alloantiserum 1124. This underscores the difficulty in typing individuals who are heterozygous for these closely related DR alleles.

DRw12 is clearly defined by the 10th IHWS monoclonal antibodies 9999 and 9050 (Table 3) as well as by DRw12 + DRw8 alloantisera (data not shown); however, no additional DR variation was detected. Since only multispecific alloantisera were available to define DRw14, it was not possible to examine DRw14 for further serologic subdivisions.

DRw6 and DR5 alleles are associated with various DRw52 alleles. TaqI/DRB3 restriction fragments at 9.6 kb and/or 12 kb were found in all DRw52-positive individuals studied and correlate with the DRw52a/b/c assignments made by T-cell clone typing (Fig. 1, Table 2). DRw13 is found in association with DRw52a, DRw52b, and DRw52c. DRw14 is found in association with DRw52a and DRw52b. DRw11 is found with DRw52b and DRw52c. DRw12 is found with

DRw52a and DRw52b. Even though the distinction between DRw52a and DRw52c could not be made using TaqI, cells expressing DRw52c carry a 16.5-kb PvuII/DRβ fragment that is not observed in cells expressing DRw52a or DRw52b (data not shown). This fragment was detected in cells that typed as DRw52c positive using a T-cell clone reagent and is found in association with haplotypes DRw11B, DRw13C and DRw13D. [An additional haplotype, DRw13H (cell 1233 in group 3, Table 3) carrying DRw13, DRw52c, DQw6 (1A76PA-positive), D- also exhibits the 16.5-kb PvuII/DRβ fragment.] Interestingly, the expression of DRw52c by more than 60% of DRw13,D- individuals and by one DRw11-positive haplotype suggests that the hypothesis that DRw52c is always associated with DRw13, Dw19 [31] only applies to some population groups. Likewise, DRw11 and DRw12 are not always associated with DRw52b.

DRA. As previously observed [32], DRA BglII fragments correlate well with DRw52 subtypes: a 4.5-kb fragment is associated with the DRw52b allele whereas a 4.2-kb fragment is associated with the DRw52a allele (Table 2). DRw52c-positive black individuals carry a 4.5-kb fragment (in haplotypes DRw13C, DRw13D, and DRw11B) while the DRw52c-positive DRw13 HTC (SLE-005, haplotype DRw13C) carries a 4.2-kb fragment. The association of DRw52c with either BglII/DRA polymorphic fragment implies that a reciprocal gene recombination between DRB3 and DRA may have generated these combinations.

DRw13 and DRw11 are associated with multiple DQ specificities. DRw13 is associated with DQw5, DQw6, and DQw7 (Table 2) in contrast to the DRw13, DQw6 association commonly observed in Caucasoids [8]. Most of the American black individuals who type as D- exhibit the less common DQ associations. Two out of 27 unrelated DRw13-positive individuals on the panel (7%) express a DQw7-associated DRw13 haplotype which has been found in 2% of DRw13-positive Caucasoids [8]. The DQw5- and DQw2-associated DRw13 haplotypes have not been reported previously. The DQw5-associated DRw13 haplotype is found in 22% of the DRw13-positive American black panel and predominates (46%) among the DRw13 individuals typed as HLA-D-. The DQw2-associated haplotype is found in 4% of the same panel. DRw14 is most frequently found in association with DQw5 on the panel as it is in the Caucasoid population [8].

In the American black cell panel, the DRw11 alleles are found associated with DQw5 and DQw7 alleles. DRw11 may also be found in association with DQw6 (cell 1553 in Table 4); however, an informative family is not available to confirm this. This haplotype (DRw11,DQw6) has been reported [HTC FPA, 10,33] and is designated haplotype DRw11E. (This haplotype was not analyzed for RFLP.) The DRw11v (Table 4, group 1) has been observed only in association with DQw7. DRw11 is also found in association with an undefined DQ allele, DQ- (cell 2009), in one family (family 011). This DQ- allele exhibits an unusual serologic pattern with DQw2 and DQw3 alloantisera in family segregation analysis (data not shown). Previously, DRw11 was thought to be primarily associated with DQw7. DRw12 is associated with DQw5 in American black individuals as it is in South African blacks [8,34]. In populations of northern European background, DRw12 is found primarily in association with DQw7 [8].

Additional DQ diversity is detected using RFLP analysis. The polymorphisms of the DQα and DQβ regions of the DRw6 and DR5 haplotypes were analyzed using the same American black families (Table 1) and HTCs as in the previous DRβ RFLP section. DQα and DQβ probes and a series of restriction enzymes were used in this study.

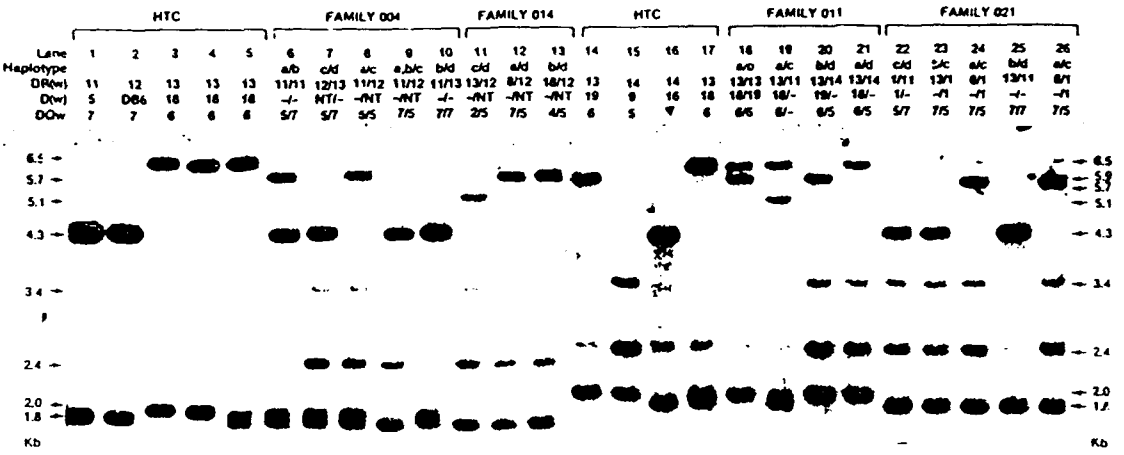

FIGURE 2 Southern hybridization analysis of DRw6/5-related haplotypes in four American black families. Genomic DNAs were digested with TaqI and probed with a DQα probe. HTCs and families are described in Fig. 1. [ARENT, a DRw13, Dw18 HTC, carried both DQA2 gene fragments (1.8 and 2.0 kb) implying heterozygosity in the DQA2 gene.]

DQα. In a comparison of the DQα-associated TaqI gene fragments among different families and HTCs (Fig. 2, Table 2), gene fragments specific for each DQ serologic specificity were detected. Two different DQw5-associated DQA1 RFLP patterns were observed. The first pattern exhibiting both 3.4- and 2.4-kb fragments is found in DQw5-positive DRw14 (haplotypes DRw14A and DRw14C), DRw12 (haplotypes DRw12B and DRw12C), and DR1 individuals (Fig. 2). The second DQw5-associated pattern consisting of a 5.9-kb fragment is associated with the DRw11-associated DQw5 (haplotype DRw11B). Two DQw6 patterns were identified. The first, a 5.9-kb fragment, found in haplotype DRw13C, is shared with the DQw5-positive DRw11 haplotype (DRw11B) suggesting the relatedness between these DQA1 alleles. A second DQw6-associated DQA1 gene fragment, a 6.5-kb fragment, is associated with DRw13A and DRw13B haplotypes.

A 4.3-kb DQw7 DQA1 gene fragment is found in all of the DRw6 and DR5 individuals and HTCs carrying DQw7. [A second pattern consisting of a 5.7-kb fragment is associated with the DRw8, DQw7 haplotype (families 014 and 021)]. The 5.1-kb fragment associated with DQw2 is also found associated with DQ- (haplotype DRw11C) although DQ- differs from DQw2 when other restriction enzymes (EcoRI, HindIII, and PstI) are used.

Based on previous studies [32], 2.0- and 1.8-kb TaqI fragments encode the nonexpressed DQA2 gene, and either one or both fragments were carried by all HTCs and individuals investigated (Fig. 2, Table 2). The RFLP patterns obtained using EcoRI, HindIII, and PstI restriction enzymes showed DQα gene fragments associated with DQw1, DQw2, and DQw3 alleles but did not show polymorphism within each DQ specificity cluster (Table 2).

DQβ. Using a DQβ probe and the BamHI restriction enzyme, polymorphism was detected at the DQβ loci (Fig. 3, Table 2). Two different DQw6-associated DQβ gene fragment patterns were identified. One pattern consisting of fragments at 3.2 and 7.5 kb is associated with DRw13A and DRw13B haplotypes. The second pattern, a single 7.5-kb fragment, is associated with the DRw13C haplo-

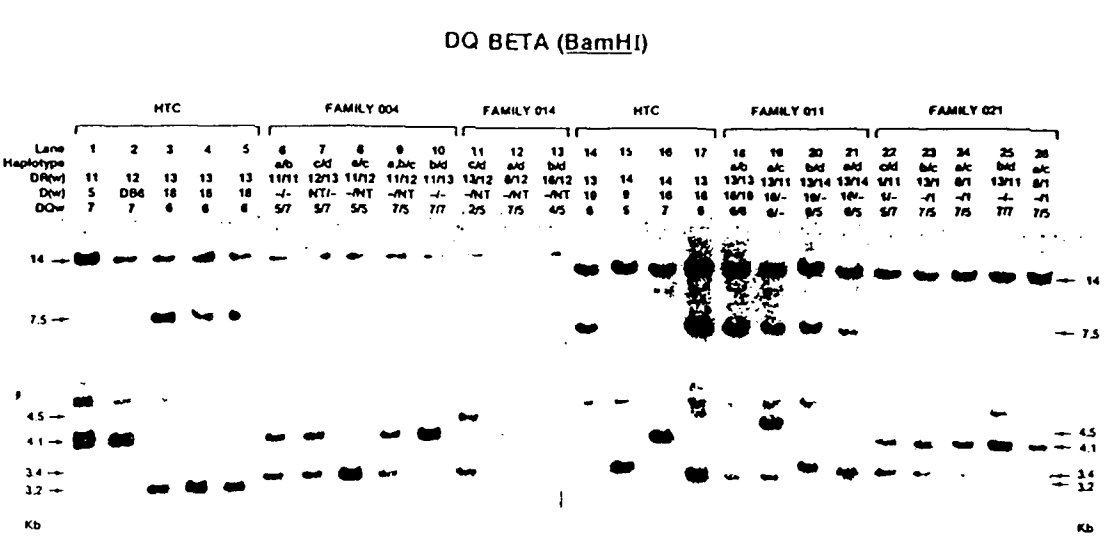

FIGURE 3 Southern hybridization analysis of DRw6/5-related haplotypes in four American black families. Genomic DNAs digested with BamHI were probed with a DQβ probe. HTCs and families are described in Fig. 1.

type. Although the possibility cannot be ruled out that these polymorphic fragments have originated from the nonexpressed DQB2 gene, this seems unlikely since the DQB2 gene appears to be conserved [35].

BamHI/DQβ gene fragments specific for the DQ-, w2, w5, and w7 serologic specificities were also detected. A 4.5-kb fragment is associated with DQw2 and DQ-, although DQ- differs from DQw2 when TaqI is used (7.2-kb TaqI fragment/DQw2 and 3.9-kb TaqI fragment/DQ-). Using TaqI, DQβ gene fragments specific for each DQ supertypic serologic specificity (DQw1, DQw2, and DQw3) were identified. However, discrimination between DQw6 (expressed by haplotype DRw13C) and DQw5 (expressed by haplotypes DRw14A, DRw14C, DRw12B, and DRw12C) alleles was not possible since both groups share a 5.7-kb DQβ fragment (Table 2). RFLP patterns using other restriction enzymes (PvuII and EcoRI) and family 004 in a pilot study did not show any additional polymorphism (data not shown).

Two DQw6 variants are also detected using alloantisera. The serologic reaction patterns for DQw1, DQw6, and DQw5 are shown in Table 3. DQw5 is defined by reactivity with alloantisera BELL, LEIBY, and 01210. DQw6 is defined by positive reactions with IHWS DQw1 sera (1153, 1162) and negative reactions with DQw5 sera. HLA-DRw13-positive cells, which type with HTCs as Dw18 or Dw19 (groups 1 and 2), are associated with DQw6. One alloantiserum, 1A76PA, reacts with DRw13, Dw18-positive cells (group 1) but not with DRw13, Dw19-positive cells (group 2). Lysostripping was employed to identify the class II molecule recognized by alloantiserum 1A76PA. Results of lysostripping using monoclonal antibodies specific for DR, DQ, DP and β-2 microglobulin indicate that alloantiserum 1A76PA reacts with DQ molecules and not with DR or DP molecules (Fig. 4). This ability to discriminate between DQw6 associated with Dw18 or Dw19 was confirmed in a larger sample (11 unrelated individuals) expressing HLA-Dw18 or Dw19 (data not shown). Several unrelated DR13,D-blacks represented by cell 1233 (DRw13, DRw52c, DQw6, D-) are also positive with alloantiserum 1A76PA defining group 3 in Table 3. This defines haplotype DRw13H. (This haplotype could not be studied by RFLP analysis because an informative family was not available.) The DQw6 subdivision parallels the split

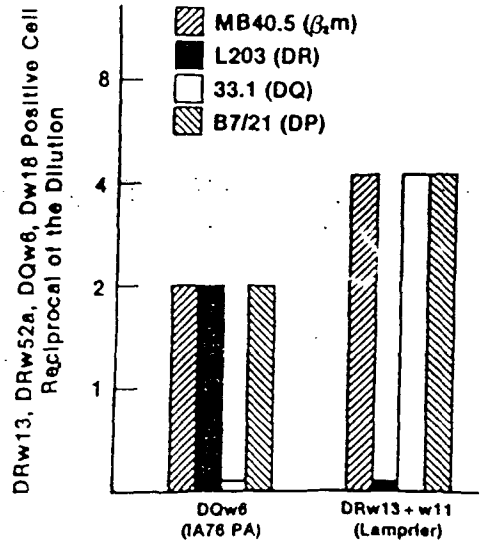

FIGURE 4 The cytotoxic titers following lysostripping with the indicated monoclonal antibodies are illustrated for a representative DRw13, DRw52a, DQw6, Dw18-positive cell and alloantiserum IA76PA. Results are also shown for a DRw13-specific alloantiserum, 1113. The monoclonal antibody MB40.5 is specific for β-2 microglobulin and will "strip" HLA-A, -B, and -C molecules from the cell surface.

defined using the DQα and DQβ probes and correlates with cDNA sequence data [36] defining two different DQw6 molecules associated with these haplotypes.

DISCUSSION

This study demonstrates the diversity of DRw6 and DR5 haplotypes in American blacks using serology, HTC typing, and RFLP analysis. The similarity between DRw13 and DRw11 DRB1 alleles in nucleotide sequence and the historical association of DRw14 and DRw12 with these alleles provide a rationale for studying DRw6 and DR5 haplotypes as a single group. In addition to the DRw6 and DR5 haplotypes, which are already well defined and represented by HTCs (haplotypes DRw13A–w13C, DRw14A–w14B, DRw11A, and DRw12A), 5 DRw13 (DRw13D–w13H), 1 DRw14, 3 DRw11 (DRw11B–w11D), and 2 DRw12 haplotypes were identified. These new haplotypes were defined in a relatively small sample size (57 DRw6- and/or DR5-positive individuals out of 105 unrelated black individuals). This level of diversity is extremely high and is the result of variability at the level of the allele and at the level of the haplotype.

Potentially, at least two new DRw13 alleles were identified in this study. Unlike DRw13 HTCs, cells expressing DRw13E, DRw13F and DRw13G haplotypes exhibit a DRw11-like 5.9-kb TaqI/DRB1 fragment (Table 2) although they serologically type as DRw13. [A similar fragment has been identified in the HTC HAG (DRw13, DQw7) using TaqI and a DRβ probe [37].] The DRw13E and DRw13F haplotypes (Table 3, group 6) show a unique pattern of serologic activity reacting with two out of seven DRw11 alloantisera in addition to DRw13 alloantisera, suggesting that they encode a new DRw13 variant (DRw13v1). Although the DRw13 serologic reactivity pattern was indistinguishable from DRw13 reference cells (Table 3), haplotype DRw13G shows the same DRA, DRB1, DRB3, DQα, and DQβ RFLP fragments as the DRw11A haplotype (HTC IDF) (Table 4). This suggests that this DRw13 allele may be a second new DRw13 variant (DRw13v2). These two DRw13 variants may have been generated by gene conversion events affecting the DRw11 haplotype and resulting in the loss of some of the DRw11 serologic determinants and the acquisition of DRw13 serologic

reactivity. Similar events have been postulated to generate the DRw17 [20] and DR'BON' [38] DRB1 alleles.

While the serologic data suggest at least two subtypes of DRw11 are present in the black population (Tables 3 and 4), no variation was observed in the DRw11 DRB1 RFLP pattern (Table 2) in this study. These DRw11 subtypes may correlate with some of the DRw11 microvariants identified by cDNA sequencing [39,40, 41]. Gene conversion may also have given rise to the DRw11v allele which exhibits a serologic reactivity pattern similar to the DRw13 variants. It is known that one DRw11 allele defined by cDNA sequencing shares the third variable region with DRw13 [39], which could produce the observed serologic reactivity pattern. Thus, the DRw13v and DRw11v alleles associated with DRw13E, DRw13F, DRw13G, and DRw11D haplotypes represent a bridge between DRw13 and DRw11 in the evolutionary pathway of the DRw52 family.

Polymorphism was not detected in the DRB1 alleles from the other DRw13 haplotypes (DRw13A–w13D) and all DRw14 haplotypes (DRw14A–w14C) using serology (Table 3, groups 1–4) and RFLP analysis (Table 2), although micropolymorphism of DRB1 has been identified by cDNA sequence analysis [20,21,39, 42]. In the DRw12A–w12C haplotypes, polymorphism was not identified in the DRB1 locus by either serology or RFLP analysis (Tables 2 and 3). This was not unexpected since only a single DRw12 DRB1 sequence has been reported [43].

DQ alleles also showed previously undescribed polymorphism. A potentially new DQ allele (DQ-) in haplotype DRw11C was identified by serologic typing and confirmed by its association with unique RFLP DQα and DQβ fragments (Table 2). Moreover, the DQw5 allele associated with haplotype DRw11B exhibits a new DQα and DQβ combination exhibiting a DQα fragment found previously in cells expressing DQw6 and a DQβ fragment previously found in cells expressing DQw5. This combination may have been generated by reciprocal recombination between DQA1 and DQB1 alleles, events postulated to generate some of the DQw3 [44] and DQw4 [25] alleles.

These DRw6 and DR5 haplotypes, specifically DRw13 and DRw11, contain a variety of combinations of DRB1 alleles with DRB3, DQA1 and DQB1 alleles. Since both DRw52 and DQ molecules may play a role in the mixed leukocyte culture defined HLA-D specificity [45,46], it is likely that the undefined HLA-D specificities detected in the DRw6 and DR5 haplotypes arise from different DR/DRw52/DQ allele combinations compared to haplotypes defined by HTCs, as well as from differences in the DRB1-encoded molecules. For example, the DRw14, DRw52a, DQw5, D- haplotype (DRw14C) encodes DRw52a in contrast to the previously described DRw14A haplotype (DRw14, DRw52b, DQw5, Dw9). Since the DRw14C haplotype shares DRB1, DQα and DQβ DNA restriction fragments with an HTC expressing the DRw14A haplotype and is serologically identical to this HTC, it is likely that the DRw52 allelic difference between the DRw14A and DRw14C haplotypes may produce the undefined HLA-D specificity found in the DRw14C haplotype. Likewise, the undefined HLA-D specificity in haplotype DRw11C may be due to the association of DRw11 with DQ- instead of the usual DQw7 allele. Haplotype DRw13D is another example where the only recognizable difference between this haplotype and the DRw13C haplotype appears to be a DQ difference (DQw5 versus DQw6). Confirmation of these predictions using cDNA sequence analysis is currently underway.

These data—the association of DRw6 and DR5 alleles with many of the DQ and DRw52 alleles—suggest that a high rate of reciprocal recombination was involved in generating these haplotypes. Thus, historic recombinations are postu-

lated to have occurred between DRA and DRB3 (e.g., haplotype DRw13C), DRB3 and DRB1 (e.g., haplotypes DRw13A/w13B and DRw12B/w12C), DRB1 and DQA1 (e.g., haplotype DRw12A/w12C), and DQA1 and DQB1 (e.g., haplotype DRw11B).

In addition to the serologic and RFLP similarities observed between DRw13 and DRw11 alleles, the high frequency of heterozygotes expressing two DRw6 and/or DR5 alleles (due to the high frequency of DRw6 and DR5 haplotypes in American blacks) increases the difficulties encountered in serologic typing of this population. This situation is compounded by the diverse combinations of class II alleles encoded by a haplotype so that a common DQ or DRw52 association cannot be used as an aid in specificity assignment. HLA typing is critical in the American black population where the high risk of hypertension frequently leads to end-stage renal disease [47], for which the therapy of choice is kidney transplantation. Therefore, precise characterization of DRw6 and DR5 haplotypes in the American black population and the development of new typing protocols utilizing multiple typing techniques is important in improving long-term graft survival in this population.

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